Tonic neurogenic inhibition of interleukin-6 secretion from murine spleen caused by opioidergic transmission

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Tonic neurogenic inhibition of interleukin-6 secretion from murine spleen caused by opioidergic transmission. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R997–R1003, 1998.—The peripheral nervous system and the immune system were shown to have neurohumoral interactions. This study extends observations that demonstrated neuronal modulation of spontaneous interleukin-6 (IL-6) secretion in the spleen by norepinephrine (NE) and β-endorphin. Spontaneous IL-6 secretion in vivo was markedly reduced by removal of macrophages with the clodronate technique. Furthermore, spontaneous IL-6 secretion was significantly inhibited at physiological concentrations of cortisol (10⁻⁷ M). In the presence of 10⁻⁷ M cortisol, addition of norepinephrine (NE; 10⁻⁵ M) and isoproterenol (10⁻⁶ and 10⁻⁵ M) significantly increased spontaneous IL-6 secretion (+20%; \( P = 0.0280 \), \( P = 0.0005 \), and \( P = 0.0050 \), respectively). In contrast, addition of β-endorphin significantly inhibited spontaneous IL-6 secretion in the presence of 10⁻⁷ M cortisol (−40%; \( P = 0.0410 \); \( 10⁻¹⁰ M, P = 0.0005 \)). To study the effect of endogenously released transmitters on spontaneous IL-6 secretion, spleen slices were electrically stimulated with 1, 5, 10, 50, and 100 Hz. Spontaneous IL-6 secretion was markedly reduced at a frequency of 10 Hz with 10⁻⁷ M cortisol present (\( P < 0.0001 \)). This indicates that the combination of nerve firing at 5–10 Hz and physiological cortisol conditions inhibits spontaneous IL-6 secretion. Inhibition of spontaneous IL-6 secretion from spleen macrophages is most probably due to a net inhibitory effect of opioidergic transmission under these conditions.

murine spleen slices; macrophages; norepinephrine; β-endorphin; inhibition of cytokines

Regional tissue inflammation is associated with invasion, activation, and proliferation of immune competent cells. Macrophages, T and B lymphocytes, dendritic cells, endothelial cells, and various other cell types are involved and produce large amounts of soluble paracrine mediators such as cytokines, which can participate in the control of local responses. In this local control system some mechanisms, such as secretion of interleukin (IL)-1 receptor antagonist (12), shedding of membrane molecules (2) and cytokine receptors (17), secretion of IL-10 (11), and downregulation of adhesion molecules (2), seem to be anti-inflammatory. Besides the local anti-inflammatory control, cytokines, which appear in the circulation, activate the hypothalamic-pituitary-adrenal (HPA) axis to increase serum cortisol levels for the control of local responses (4, 8). Another control mechanism is the activation of the hypothalamic-autonomic nervous system (HANS) axis, which results in increased nerve firing rates, for example, in splenic nerves (22), and increased turnover of sympathetic transmitters in presynaptic nerve terminals (1, 32, 34). Activation of the central nervous system by cytokines significantly modulates cellular immune responses, which is independent of humoral, blood-mediated mechanisms (10, 30, 31). This may indicate an important role of other nonhumoral mechanisms such as the HANS axis in the local control of immune responses. Normally, sympathetic nerves have a firing rate of ~5–10 Hz, which is the spontaneous rhythm of the sympathetic autonomic nervous system (22, 23). Under these basal conditions, the synaptic norepinephrine (NE) concentration is ~0.15 µmol/l (6). During systemic inflammation, the nerve firing rate reaches up to 100 Hz (22) and, hence, endogenous transmitters such as NE reach high concentrations of ~10 µmol/l in the synaptic cleft (5). Both central nervous mechanisms, HPA axis and HANS axis, act in parallel to systemically control production and secretion of cytokines at local tissue sites or in lymphoid organs.

In earlier studies, we were able to demonstrate a new superfusion technique to investigate the dose interaction between autonomic presynaptic nerve terminals (and their transmitters) and immune-competent cells in the murine spleen (27–29). These studies demonstrated spontaneous IL-6 secretion from murine spleen slices directly after transfer into microsuperfusion chambers. The spontaneous IL-6 secretion was partly inhibited by electrically released endogenous NE and endogenous opioids (27). The aim of the present study was to analyze the role of macrophages for the spontaneous IL-6 secretion and to investigate more closely the conditions for spontaneous IL-6 secretion. Glucocorticoids, which are the most important humoral modulators secreted after activation of the HPA axis during inflammation, were added to examine spontaneous IL-6 secretion under conditions that come closer to the in vivo situation. Furthermore, it was the goal to examine the frequency-response curve during electrical stimulation to simulate effects of different nerve firing rates found during inflammation at the splenic nerve.

This study demonstrates that macrophages were involved in the early spontaneous IL-6 secretion in murine spleen. Electrical stimulation of spleen slices with various stimulation frequencies eliminated spontaneous IL-6 secretion. With glucocorticoids, α-adrenergic and β-adrenergic agonists stimulated IL-6 secretion, whereas β-endorphin markedly inhibited IL-6 secretion. This indicates that the HANS axis, most
probably by basal opioidergic transmission, leads to a net inhibition of spontaneous IL-6 secretion from the spleen in the presence of glucocorticoids.

**MATERIALS AND METHODS**

Animals, superfusion medium, and preparation of the tissue. Female NMRI mice (8–10 wk; 26–34 g; Charles River, Sulzfeld, Germany) were used. Spleens were removed after cervical dislocation. The spleen was kept on ice in culture medium for 10 min (RPMI 1640, 25 mM HEPES, 5% FCS, 30 μM mercaptoethanol, 0.57 mM ascorbic acid, 1.3 mM calcium, 100 IU/ml penicillin, and 100 μg/ml streptomycin; all additions from Sigma, Munich, Germany) before it was cut into 0.35-mm-thick slices with a tissue chopper (Mickle Laboratory, Gomshall, England) and washed carefully (direction of cutting at a right angle to the longitudinal axis of the spleen).

Superfusion protocol and standardization. Spleen slices were transferred to minisuperfusion chambers with a volume of 80 μl and equipped with two perforated gold disc electrodes forming the bottom and the top of each chamber, respectively (29). Superfusion was performed for 8 h at a temperature of 37°C and a flow rate of 66 μl/min (one slice per chamber, 24 chambers in parallel). During the first 4 h of the superfusion period, all slices were superfused with culture medium with 10−7 M cortisol but without further additional drugs. Between the 225th and 240th minute, superfusate was collected to determine IL-6 (pg/ml). ELISA technique, see IL-6 determination). During the second part of the superfusion period (hours 4–8), drugs or electrical stimulation were applied to modulate IL-6 secretion (27, 28). Between the 465th and 480th min, superfusate was again collected to determine IL-6.

The dimensionless ratio ω = 100 × (IL-6 at 8 h/IL-6 at 4 h) was used to standardize the IL-6 secretion of the various slices (27, 28).

Electrical stimulation and reagents. To come closer to the in vivo situation, certain studies were done with cortisol (10−7 M hydrocortisone, Sigma) to examine spontaneous IL-6 secretion. We used cortisol instead of corticosterone because cortisol uses the same glucocorticoid receptor with comparable affinity (16) and both hormones have comparable half-lives in blood (cortisol vs. corticosterone: 1.4–3 h vs. 0.9–1.6 h; Ref. 15).

To simulate the effect of different nerve firing rates, spleen slices were electrically stimulated with various stimulation frequencies between the 260th and 480th minute (n = 12 control vs. n = 12 electrical stimulations). Thirty bursts of monophasic rectangular pulses, with an interval of 100 s between successive bursts, with various stimulation frequencies of 1, 5, 10, 50, and 100 Hz were used (50, 250, 500, 2,500, and 5,000 pulses/burst; width of the pulse: 2 ms; constant current of the pulse: 36 mA). As in earlier studies (27–29), the electrical current used was optimal for the release of endogenous presynaptic transmitters.

The naturally occurring transmitter NE (Research Biochemicals International [RBI], Natick, MA), the β12-adrenergic agonist isoproterenol (Sigma), the α2-adrenergic agonist methoxamine (Sigma), the α2-adrenergic agonist p-aminodiodiphenyl (RBI), and the mostly μ-opiergic naturally occurring transmitter β-endorphin (Sigma) were added in various concentrations between the 240th and 480th minute (n = 12 control vs. n = 12 substance). Lipopolysaccharide (LPS) from Salmonella typhimurium was purchased from Sigma. In experiments with added endotoxin, LPS was vigorously sonicated shortly before use.

Experiments with clodronate liposomes. Clodronate (dichloromethylene diphosphonate) liposomes were used as described previously (33). Briefly, 300 μl of clodronate liposomes were intravenously injected into NMRI mice. Three days after the injection, spleens were removed and slices were prepared. The slices were then transferred to microsuperfusion chambers, and the spontaneous secretion of IL-6 was observed for 10 h without cortisol in the superfusion medium. Because mice survive clodronate treatment without signs of a disease, it is thought that viability of tissue slices is not markedly affected by the treatment. Clodronate was a gift of Boehringer Mannheim, Mannheim, Germany.

IL-6 determination. Murine IL-6 in superfusate fractions was determined by ELISA (Endogen, Boston, MA). Sensitivity was <16 pg/ml. The assay ranges from 15–2,450 pg IL-6/ml. Intra-assay and interassay coefficients of variation were below 10%. The detection limit of the IL-6 ELISA was ~15–20 pg/ml.

Presentation of the data and statistical analysis. All data are given as means ± SE; n = number of observations; one observation = one slice. Using 24 chambers, 24 slices in one experiment of one mouse were investigated. In experiments with electrical stimulation or an added substance, two different conditions were investigated: 1) 12 control slices and 2) 12 slices with electrical stimulation or an indicated drug concentration. However, because of technical problems, such as loss of a slice or insufficient superfusion flow in a single pumping tube, the number of observations for each condition is not always a multiple of twelve. Because the average of one experiment varies from mouse to mouse, the effects are demonstrated in percent of control (see legends to Figs. 2, 3, and 5–8). One-way ANOVA (SPSS/PC+ Advanced Statistics V4.0.1; SPSS, Chicago, IL) was used for the determination of statistical significance, and P < 0.05 was the significance level.

**RESULTS**

Effect of macrophage depletion on IL-6 secretion by spleen slices. Macrophages phagocytize clodronate liposomes, which leads to the death and depletion of macrophages, which has been repeatedly confirmed both ultrastructurally and by loss of macrophas specific markers in liver and spleen (26, reviewed in Ref. 33). Lymphocytes, granulocytes, and dendritic cells cannot be depleted by the clodronate liposome technique (9, 21, 25). To determine the role of macrophages for the spontaneous IL-6 secretion, they were selectively eliminated by the intravenous clodronate liposome technique (9, 21, 25, 26, 33). Compared with untreated mice, 3 days after intravenous clodronate liposome injection, spleen slices from treated mice (n = 2) did not release IL-6 (P for the difference at all time points compared with untreated mice <0.0001, Fig. 1). IL-6 concentration in the superfusion medium was below the detection limit of ~15 pg/ml at 2, 4, 6, 8, and 10 h (Fig. 1).

Effect of cortisol on spontaneous IL-6 secretion. Because cortisol plays an important role in the HPA axis during systemic inflammation, it was used to simulate in vivo conditions. We used cortisol instead of corticosterone because cortisol uses the same glucocorticoid receptor with comparable affinity (16) and both hormones have comparable half-lives in blood (cortisol vs. corticosterone: 1.4–3 h vs. 0.9–1.6 h; Ref. 15). Spleen slices from untreated mice were transferred to microsuperfusion chambers and superfused with various con-
centrations of cortisol between the 240th and 480th minute. The concentration-response curve is given in Fig. 2. The concentration of $10^{-7}$ M cortisol was the IC$_{50}$ ($P = 0.003$); maximal inhibition of IL-6 secretion was found at a concentration of $10^{-6}$ and $10^{-5}$ M ($P < 0.0001$, Fig. 2). The concentration of $1 \times 10^{-7}$ M was used for further experiments because the normal serum concentration of cortisol in mice and men has been found to be $0.5 - 1.5 \times 10^{-7}$ M (3, 14).

Effect of LPS on spontaneous IL-6 secretion. Despite an extensive washing procedure (600 ml Extran; Merck, Darmstadt, Germany; 600 ml ethanol 40%, 600 ml 0.2 N HCl, 1,000 ml aqua dest) after the end of each experiment and administration of 100 IU/ml penicillin and 100 µg/ml streptomycin throughout the experiments, the superfusion apparatus cannot be kept sterile. To demonstrate the influence of LPS on spontaneous IL-6 secretion, a concentration-response study was performed. As shown in Fig. 3, LPS higher than 100 ng/ml led to a dose-dependent increase of IL-6 secretion.

Short-term and long-term influence of cortisol or electrical stimulation on IL-6 secretion. To demonstrate the short-term and long-term influence of cortisol or electrical stimulation on IL-6 secretion, the following experiments were performed. In all experiments, spleen slices of untreated mice were transferred to microsuperfusion chambers and superfused for 9 h. In the first experiment, cortisol was added for 120 min (short term) between the 3rd and 5th hour of superfusion, which delayed the increase of IL-6 secretion (Fig. 4B) compared with control (Fig. 4A). In another experiment, the continuous administration of cortisol between the 3rd and 9th hour (long term) led to a long-lasting and increasing inhibition of IL-6 secretion (Fig. 4C) compared with control (Fig. 4A). Furthermore, a short-term electrical stimulation between the 3rd and 5th hour of superfusion (2 bursts of monophasic rectangular pulses: 2 ms, 1 Hz, 43 mA, 1,500 pulses/burst) delayed and inhibited the increase of IL-6 secretion (Fig. 4D) compared with control (Fig. 4A). A long-term electrical stimulation (10 bursts of monophasic rectangular pulses: 2 ms, 1 Hz, 43 mA, 1,500 pulses/burst) between the 3rd and 9th hour of superfusion abolished IL-6 secretion between the 5th and 9th hour (data not shown).

Effect of electrical stimulation with various stimulation frequencies on spontaneous IL-6 secretion. Nor-

Fig. 1. Effect of clodronate liposomes on spontaneous interleukin (IL)-6 secretion. Two mice were treated with 300 µl clodronate liposomes intravenously to deplete macrophages from the spleen. After 3 days, spleens were removed and spleen slices were transferred to microsuperfusion chambers and superfused for 10 h at 37°C. IL-6 was measured by ELISA. For each condition, a total of $n = 24$ slices from 2 different mice was used.

Fig. 2. Effect of cortisol on spontaneous IL-6 secretion. Spleen slices from untreated mice were transferred to microsuperfusion chambers and superfused for 240 min with medium without cortisol [control (Co)]. Cortisol in indicated concentrations was added to the superfusion medium between 240 min and 480 min. IL-6 was measured by ELISA at the 4th and 8th hours. Data are given in mean percent of control ± SE. Average $\phi$ of the control was $100 \times \{156.4 \text{ (pg/ml)/123.2 (pg/ml)}\} = 126.9 \pm 4.3$ ($= 100\%$). Numbers of slices investigated for each concentration are given in the bars.

Fig. 3. Effect of lipopolysaccharide on spontaneous IL-6 secretion. Spleen slices from untreated mice were transferred to microsuperfusion chambers and superfused for 240 min with medium without cortisol. Lipopolysaccharide in indicated concentrations was added to the superfusion medium between 240 min and 480 min (no cortisol present). IL-6 was measured by ELISA at the 4th and 8th hour. Data are given in mean percent of control ± SE. Average $\phi$ of the control was $100 \times \{143.4 \text{ (pg/ml)/128.1 (pg/ml)}\} = 111.9 \pm 2.3$ ($= 100\%$). Numbers of slices investigated for each concentration are given in the bars.
ment. During high nerve firing rates, an elevated NE concentration of up to $10^{-5}$ M has been found in the synaptic cleft (5). With respect to presynaptically located peptidergic transmitters, it can be expected that they are also released in large amounts during high compared with low nerve firing rates (e.g., neuropeptide Y; Ref. 20). From previous studies in the absence of cortisol (27–29), the effects of endogenous NE and β-endorphin on spontaneous IL-6 secretion were known. To determine whether or not the inhibition of IL-6 secretion by electrical stimulation could be mediated by NE or β-endorphin in the presence of $10^{-7}$ M cortisol, spleen slices of untreated mice were superfused between the 240th and 480th minute with different concentrations of endogenous transmitters and defined receptor agonists.

At a concentration of $10^{-5}$ M, NE significantly increased IL-6 secretion from spleen slices ($P = 0.005$; Fig. 6). This was also demonstrated for the $\beta_2$-adrenergic agonist isoproterenol at $10^{-6}$ M ($P = 0.0005$; Fig. 6) and $10^{-5}$ M ($P = 0.0281$; Fig. 6). The concentration-response curve for isoproterenol was bell-shaped, with a maximum at $10^{-6}$ M (Fig. 6).

In contrast to our previous results in the absence of cortisol (27), p-aminoindoline significantly increased IL-6 secretion at $10^{-8}$ M ($P = 0.0492$; Fig. 7) and $10^{-7}$ M ($P = 0.0081$; Fig. 7). The concentration-response curve was bell-shaped with a maximum at $10^{-7}$ M, which was also shown to be the most effective concentration in our earlier studies (27). The $\alpha_1$-adrenergic agonist methoxamine had no effect on spontaneous IL-6 secretion at various concentrations ($10^{-9}$–$10^{-6}$ M) (data not shown).

As we have shown previously in the absence of cortisol (27), in the presence of $10^{-7}$ M cortisol β-endorphin significantly inhibited spontaneous IL-6 secretion at $10^{-11}$ M ($P = 0.041$; Fig. 8) and $10^{-10}$ M ($P = 0.0102$; Fig. 8).

**Fig. 4.** Effect of short- and long-lasting modulation of IL-6 secretion by cortisol and electrical stimulation. Spleen slices of untreated mice were transferred to microsuperfusion chambers and superfused with superfusion medium for 9 h in 2 different experiments. A: control condition ($n = 10$ slices); B: short-term cortisol ($10^{-6}$ M) administration between the 3rd and 5th hour of superfusion ($n = 10$ slices). C: long-term cortisol ($10^{-6}$ M) administration between the 3rd and 9th hour of superfusion ($n = 8$ slices). D: short-term electrical stimulation (3 trains of monophasic rectangular pulses: 2 ms, 1 Hz, 43 mA, 1,500 pulses/train) between the 3rd and 5th hour of superfusion ($n = 8$ slices). Black horizontal bars in A-D indicate period of modulation with cortisol or electrical stimulation. All data are given as means ± SE (pg/ml).

**Fig. 5.** Effect of stimulation frequency on spontaneous IL-6 secretion. Spleen slices of untreated mice were transferred to microsuperfusion chambers and superfused with (hatched bars) or without (open bars) $10^{-7}$ M cortisol in superfusion medium for 480 min. Between 260 min and 480 min, the spleen slices were electrically stimulated with the indicated stimulation frequencies. Shaded area indicates normal spontaneous sympathetic nerve firing rate in vivo. IL-6 was measured by ELISA at the 4th and 8th hours. IL-6 cut-off is shown as a broken horizontal line. Data are given in mean percent of control ± SE. Average $\phi$ of the control without cortisol was $100 \times [162.1 \text{ pg/ml/133.4 (pg/ml)]} = 121.5 \pm 3.3 (= 100\%)$. Numbers of slices investigated for each concentration are given in the bars.
endorphin inhibited IL-6 secretion by 40% (Fig. 6), with the NE-induced increase in IL-6 of 20% (Fig. 8), β-endorphin inhibited IL-6 secretion by 40% (Fig. 6), and with respect to isoproterenol, 20% (Fig. 6), β-endorphin inhibited IL-6 secretion by 40% (Fig. 8).

The concentration-response curve was U-shaped with a maximal inhibition at 10^{-10} M, which had been shown to be the most effective inhibiting concentration in our earlier studies (27). Compared with the NE-induced increase in IL-6 of ~20% (Fig. 6), β-endorphin inhibited IL-6 secretion by ~40% (Fig. 8).

DISCUSSION

Several groups have proposed an involvement of the autonomic nervous system in the control of immune functions in lymphoid and other organs (1, 4, 7, 13, 19, 22, 30–32, 34). This hypothesis is based on 1) the close anatomic interaction of presynaptic nerve terminals and immune-competent cells such as macrophages in the spleen; 2) the presence of various transmitter receptors on immune-competent cells, such as macrophages; and 3) the control in lymphoid organs of the sympathetic nerve firing rate or transmitter content by immune stimuli, such as centrally or peripherally administered cytokines. With the recent introduction of a modified superfusion technique with microchambers using electrical stimulation, we demonstrated the close functional interaction of autonomic presynaptic nerve terminals and immune-competent cells (27–29). In these recent studies, spleen slices transferred to the microsuperfusion chambers spontaneously secreted large amounts of IL-6. Because it was shown that after cell culture initiation maximal IL-6 mRNA expression in monocytes and T lymphocytes was reached at 5 and 24–48 h, respectively (18), it was assumed that early IL-6 secretion in our studies was mainly derived from macrophages. The intravenous application of clodronate liposomesabolished spontaneous IL-6 secretion. Because depletion of macrophages using clodronate liposomes has been repeatedly confirmed both ultrastructurally and by loss of macrophage-specific markers (9, 21, 25, 26, 33), this effect indicates the prominent role of macrophages for the spontaneous IL-6 production in the spleen. Lymphocytes (9), granulocytes (21), and dendritic cells (25) as sources of spontaneous splenic IL-6 secretion in our tissue slice model are largely excluded because these cells cannot be depleted by the clodronate liposome technique.
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In our model, this spontaneous IL-6 secretion may be due to 1) tissue injury and subsequent release of stimuli, 2) LPS contamination of the medium, 3) absence of normally present inhibiting humoral factors such as cortisol and others, or 4) absence of a tonic neural mediation inhibition of IL-6 secretion. The contamination with LPS does not seem to be the main factor for spontaneous IL-6 secretion because LPS concentrations of 100–500 ng/ml led to an obvious increase of IL-6 secretion. This indicates that the endotoxin concentration in our experiments was certainly not much above 100 ng/ml, which is a concentration that did not markedly stimulate in vitro NMRI mouse splenocytes for IL-6 secretion. To more closely investigate the spontaneous IL-6 secretion, glucocorticoids were used to simulate in vivo conditions because glucocorticoids act as major inhibitors of cytokine production. The addition of cortisol at 10⁻⁶ M led to a 50% inhibition of the spontaneous IL-6 secretion. During systemic inflammation, cortisol is upregulated to ~10⁻⁶ to 10⁻⁵ M (4). Hence, cortisol is most likely able to inhibit stimulated IL-6 secretion. Short-term modulation with cortisol at 10⁻⁶ M and electrical stimulation both delayed and/or inhibited IL-6 secretion, which clearly indicates the transient inhibiting effects of glucocorticoids and (electrically) released endogenous neurotransmitters.

With the use of microsperfusion chambers, electrical field stimulation of spleen slices leads to neurotransmitter release (29) and to a marked inhibition of spontaneous IL-6 secretion, which can be significantly attenuated by competitive transmitter antagonists such as naloxone or phenotlamine (27). From this point of view, we wanted to study the role of released endogenous neurotransmitters for the spontaneous IL-6 secretion using various nerve firing rates. Increase of the stimulation frequency, for example during inflammatory stimuli (22), leads to high concentrations of endogenous presynaptically released transmitters (5, 6, 20). Moreover, the ratio between several released transmitters such as NE and neuropeptide Y changes with different nerve firing rates (20). It was suggested that exocytosis of material such as neuropeptides (for example, neuropeptide Y) from large vesicles is increased at higher frequencies (>10 Hz; Ref. 20) in relation to NE from small vesicles. During inflammation, the cytokine-stimulated brain modulates the nerve firing rate. The normal nerve firing rate is ~5–10 Hz (22, 23), whereas during inflammatory stimuli the nerve firing rate increases up to 100 Hz (22). Under conditions with high firing rates, the NE concentration in the synaptic cleft reaches high concentrations of ~10⁻⁵ M (5). For this reason, the frequency-response curve for IL-6 secretion under conditions with and without cortisol was studied. The data clearly indicate that spontaneous IL-6 secretion can be modulated by different nerve firing rates. At 5–10 Hz, spontaneous IL-6 secretion was significantly reduced in the presence of cortisol. Further experiments with exogenous agonists revealed that β-endorphin is a major inhibitor of IL-6 secretion in the presence of cortisol. In contrast, NE slightly increased the spontaneous IL-6 secretion, which was due to β-adrenergic and α₁-adrenergic mechanisms in the presence of 10⁻⁷ M cortisol. Because NE and β-endorphin are secreted in parallel (27) and the increase in IL-6 induced by NE (~20%) is less marked compared with the inhibition of IL-6 secretion induced by β-endorphin (~40%), it is conceivable with reference to our earlier studies (27) that electrical stimulation leads to a net inhibition of IL-6 secretion in the presence of cortisol. It was demonstrated that naloxone only partially antagonized the electrically induced inhibition of IL-6 secretion (27). Hence, it may be possible that endogenously released β-endorphin acts via the naloxone-insensitive β-endorphin receptor (35). Furthermore, the agonist-induced receptor-mediated dose–response curves show an optimum with a U-shaped or bell-shaped form. These response curves can occur when a ligand acts on more than one receptor on the same cell or on a different cell. Ligation of the same receptor on different cell types using a common readout such as macrophage IL-6 can produce optima. In this study, we used spleen slices so that all of the above-mentioned events can take place.

In conclusion, macrophages are responsible for the spontaneous IL-6 release in the murine spleen. A recent clinical study suggested that there may be spontaneous production of IL-6 (24). This spontaneous IL-6 secretion may indicate a low-level activation of splenic immune-competent cells, such as macrophages, which are involved in the phagocytosis of aged erythrocytes and other cellular material in the healthy spleen. Cortisol and the basal nerve firing with ongoing release of sympathetic transmitters downregulate this spontaneous production of IL-6 mainly via opioidergic mechanisms.

Perspectives

Communication between the neuroendocrine and immune system is commonly associated with release of humoral factors such as cortisol and epinephrine of the HPA axis. During a limited inflammatory response, cytokines are locally released and may become important humoral stimuli to activate the HPA axis. It was thought that activation of the HPA axis is necessary to dampen inflammatory responses. Besides the HPA axis, a neuronal control system in lymphoid organs also plays an important role in this feedback inhibition of cytokine secretion during inflammation. However, under physiological conditions without any inflammatory stimulus, explanted splenic tissue slices still produce measurable amounts of IL-6. This study extends our earlier observations and indicates that inhibition of unstimulated IL-6 secretion in spleen slices in the presence of cortisol is caused by continuous neurotransmission. Under physiological conditions, splenic macrophages are locally balanced by neurogenic inhibition and phagocytic stimulation. This tonic neurogenic inhibition is extensively upregulated during a systemic immune response when the firing rate and the neurotransmitter release increase. During systemic inflammation, HPA axis and HANS axis are optional inhibitory systems that are used to reduce excessive immune responses. From the present point of view, in addition
to the HPA axis the HANS axis may be a suitable target to therapeutically control systemic inflammation.

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